X-ray Crystallographic Studies of *S. aureus* MurB

The enzyme catalyst MurB is present in both Gram-negative bacteria, such as *Escherichia coli,* and Gram-positive bacteria, such as *Staphylococcus aureus*. The structural characterizations of *S. aureus* MurB can serve as a basis for development of novel inhibitors using structure-based drug design. With this focus, the x-ray crystal structure of *S. aureus* MurB was solved by selenomethionine multiple-wavelength anomalous dispersion using synchrotron radiation at 2.3-Å resolution. The structure reveals a protein fold similar to the one observed in the *E. coli* MurB structure.

The increasing occurrence of antibiotic-resistant bacterial infections has spurred efforts to identify new classes of antibacterial agents. While most antibiotics to date have been developed by screening natural products, the recent emergence of whole genome sequences for a number of pathogenic bacteria has provided rapid access to molecular-based approaches in target identification and screening. As a result, targets from essential biochemical processes, such as bacterial cell wall biosynthesis, have become the focus of intensive pharmaceutical research. Our efforts at Pharmacia have incorporated x-ray crystallography as part of the drug discovery process to provide information on the structures of protein targets from bacterial cell wall biosynthesis [1,2].

Because of the cell wall's fundamental role in providing osmotic stability and maintaining cellular integrity, its synthesis has been an excellent target for antibiotics. One of the key components of the cell wall is a series of cross-linked disaccharide-polypeptide units that give the wall its osmotic strength. Although historical inhibitors of peptidoglycan biosynthesis have targeted the cross-linking step between the peptide strands, recent efforts have been directed at inhibiting the synthesis of the sugar polymer and the elongation of the peptide chain.

Synthesis of peptidoglycan is a multistep process that begins with the generation of a unique sugar, UDP-N-acetylmuramic acid. Formation of UDP-N-acetylmuramic acid occurs by a two-enzyme process catalyzed by MurA and MurB (Fig. 1). MurA transfers the enolpyruvyl group from phosphoenolpyruvate to the 3′-hydroxyl of UDP-N-acetylglycosamine (the other sugar that makes up the disaccharide of peptidoglycan). The conversion is completed by the enzyme MurB, which catalyzes the formation of the lactyl ether of N-acetylmuramic acid by reducing the enolpyruvyl moiety of the substrate with one equivalent of NADPH [3]. This lactyl ether stem of muramic acid serves as the molecular connector between the disaccharide and peptide components, thereby ensuring the structural integrity of the peptidoglycan. MurB is present in both Gram- negative bacteria, such as *Escherichia coli*, and Gram-positive bacteria, such as *Staphylococcus aureus*. While many groups have studied peptidoglycan biosynthetic enzymes for *E. coli*, little work has been conducted on this pathway in a clinically relevant Gram-positive organism. Our research focused on characterizing the x-ray crystal structure of MurB from *S. aureus* and comparing it to the previously reported *E. coli* MurB structure [4] with the intention of using information from the structure of the *S. aureus* protein to guide the development of clinically relevant inhibitors.

The x-ray crystal structure of *S. aureus* MurB

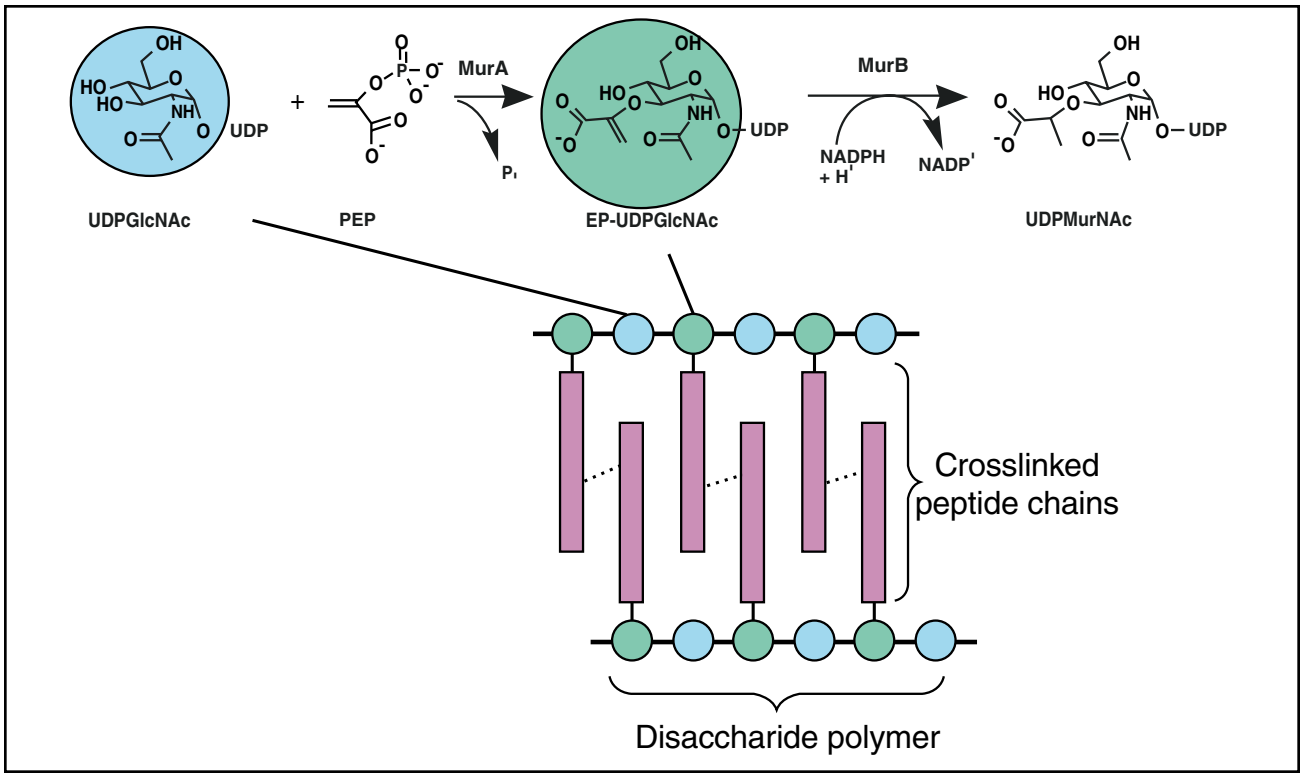

FIG. 1. Schematic view of the peptidoglycan polymer and the two-step formation of UDP-N-acetylmuramic acid catalyzed by MurA and MurB.

was solved by selenomethionine multiple-wavelength anomalous dispersion using synchrotron radiation at IMCA-CAT beamline 17-ID. Figure 2 shows crystals of *S. aureus* MurB. Selenomethionine-incorporated protein provided the heavy atoms necessary to solve the structure using tunable synchrotron radiation. Three data sets collected at the inflection point of the absorption edge, the peak of the absorption edge, and a remote point provided anomalous and dispersive differences to solve the structure at 2.3-Å resolution.

The structure of *S. aureus* MurB reveals a protein fold similar to the one observed in the *E. coli* MurB structure. The observation of a related fold is consistent with sequence alignments, which reveal an overall 22% identity and 30% similarity between the amino acid residues. The enzyme structure comprises three domains, each of which contains alpha helices and beta sheets. The first two domains form the binding site for a bound flavin adenine dinucleotide (FAD) cofactor, while the third domain comprises the substrate-binding domain. The substrate-binding domain facilitates binding of NADPH first, followed by transfer of hydride to the FAD cofactor. With the subsequent release of the $NADP^+$ product, the enolpyruvyl-UDP-*N*-acetylglycosamine (EP-UDPGlcNAc) binds the substrate domain and accepts the hydride from the reduced FAD cofactor ($FADH_2$) to produce UDP-*N*-acetylmuramic acid.

The *S. aureus* MurB protein structure does reveal several key distinctions between the two

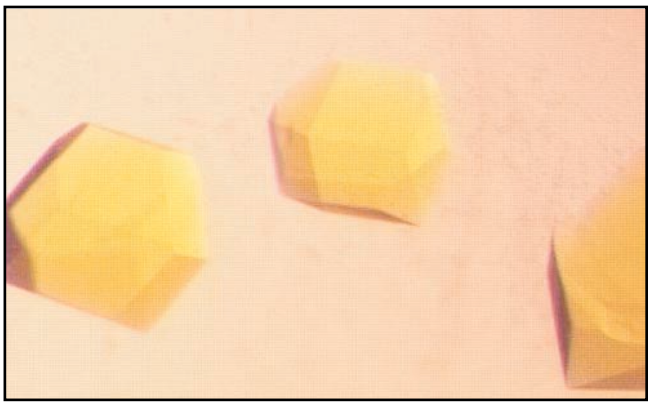

FIG. 2. Crystals of S. aureus MurB. The bound FAD cofactor gives the crystals their yellow color.

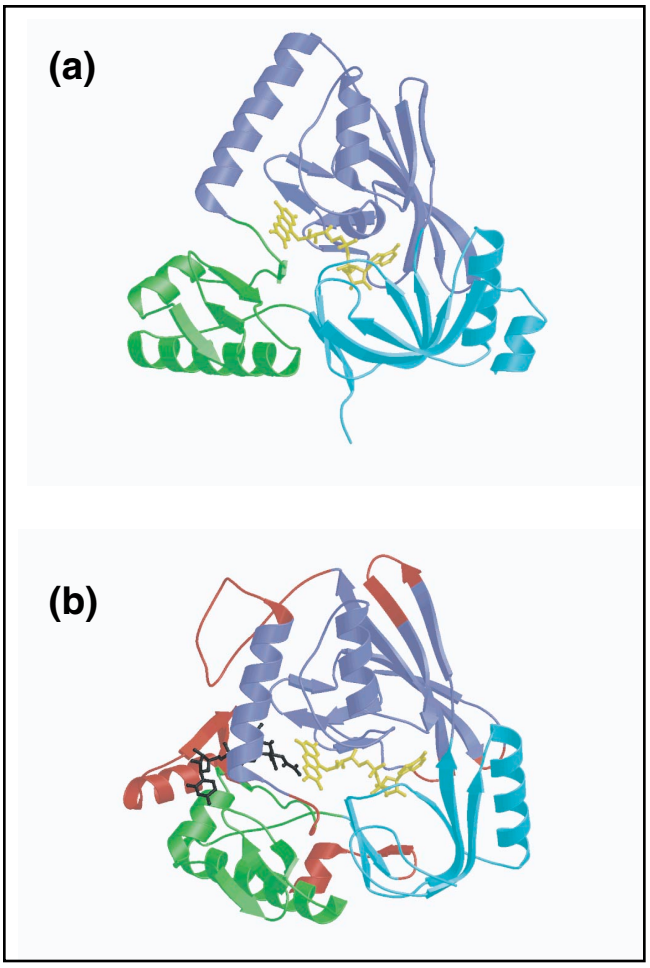

FIG. 3. Overview of the (a) S. aureus *and (b)* E. coli *MurB structures. Domains 1 and 2 (cyan and blue) comprise the FAD-binding domain while domain 3 (green) is the substrate-binding domain. The bound FAD cofactor is shown with stick bonds in yellow, and the EP-UDPGlcNAc substrate (in the* E. coli *structure) is shown with black stick bonds. Sections of the* E. coli *MurB structure that are absent in the* S. aureus *MurB structure are shown in red.(Reprinted by permission from "A Structural Variation for MurB: X-ray Crystal Structure of Staphylococcus aureus UDP-N-acetylenolpyruvylglucosamine Reductase (MurB)," Biochemistry* **40***, 2340-2350 [2001]. Copyright © 2001 American Chemical Society.)*

species. Several loops and alpha helices observed in the *E. coli* MurB structure are not present in the *S. aureus* MurB structure (Fig. 3). Although insertions and deletions are not unusual when comparing the same protein from different species, these deletions significantly alter the substrate-binding domain of the *S. aureus* MurB enzyme. For instance, prior structural data for the *E. coli* MurB enzyme [5] revealed a critical tyrosine residue (Tyr-190) that undergoes a significant rotation in order to interact with the substrate, EP-UDPGlcNAc. *S. aureus* MurB lacks this tyrosine residue and must compensate with other nearby residues to provide optimal binding for the substrate. In addition, a split βαββ fold that is part of the *E. coli* MurB substrate-binding domain is absent from the *S. aureus* MurB structure. The loss of these two structural components results in a simpler protein structure for the *S. aureus* MurB enzyme.

Despite apparent differences in the binding of the substrate, the point of action of catalysis is well conserved in the structure of *S. aureus* MurB. Prior studies of *E. coli* MurB showed that the carbanion intermediate on the enolpyruvyl group (generated by the addition of a hydride from the flavin cofactor) could be stabilized by two residues—Arg-159 and Glu-325. Analogous residues Arg—188 and Glu-308—are found in the *S. aureus* MurB structure. In addition, the active site serine that aids the quenching of the carbanion intermediate (*E. coli* MurB Ser-229) is also present in *S. aureus* MurB (Ser-238).

Sequence alignment of MurB sequences from other bacterial species reveals that the *E. coli* and *S. aureus* MurB are representative of two structural classes of MurBs. The first class, type I MurB, binds the EP-UDPGlcNAc substrate using the Tyrosine-190 loop and the split βαββ fold. Bacterial species in this class include *E. coli*, *H. influenzae*, *S. typhimurium*, and *B. pertussis*. The second class is typified by the structure observed in *S. aureus* MurB where this tyrosine loop and the split βαββ fold are not present. Other members of class II MurB include *H. pylori*, *A. aeolicus*, *B. subtilis*, *B. burgdorferi*, *C. pneumoniae*, and *R. prowazekii*.

The solution of the x-ray structure of *S. aureus* MurB reveals that important differences can exist among functionally equivalent proteins of various bacterial species. Such differences underscore the importance of conducting direct structural analyses when studying related enzymes. The structural characterizations of *S. aureus* MurB can now serve as a basis for development of novel inhibitors using structure-based drug design—an iterative process that involves chemical synthesis of new inhibitors

and crystallization of protein-inhibitor complexes in order to improve the potency and selectivity of the inhibitor.

Principal publication: "A Structural Variation for MurB: X-ray Crystal Structure of *Staphylococcus aureus* UDP-*N*-acetylenolpyruvylglucosamine Reductase (MurB)," Biochemistry **40**, 2340-2350 (2001).

References

[1] T.E. Benson, M.S. Harris, G.H. Choi, J.I. Cialdella, J.T. Herberg, J.P. Martin, and E.T. Baldwin, Biochemistry **40**, 2340-2350 (2001).

[2] M.S. Harris, J.T. Herberg, J.I. Cialdella, J.P Martin, T.E. Benson, G.H. Choi, and E.T. Baldwin, Acta Crystallogr. Sect. D **57**, 1032-1035 (2001).

[3] T.E. Benson, J.L. Marquardt, A.C. Marquardt, FA. Etzkorn, and C.T. Walsh, Biochemistry **32**, 2024-2030 (1993).

[4] T.E. Benson, D.J. Filman, C.T. Walsh, and J.M. Hogle, Nature Structural Biology **2** 644-653 (1995).

[5] T.E. Benson, C.T. Walsh, and J.M. Hogle, Structure **4**, 47-54 (1996).

T. E. Benson, M. S. Harris, J. I. Cialdella, J. T. Herberg, J. P. Martin, E. T. Baldwin

The Pharmacia Corporation, Kalamazoo, MI, U.S.A.